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FLOW-BASED DETECTION OF BAR CODED PARTICLES

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ABSTRACT

We have developed methods for flow control, electric field alignment, and readout of colloidal Nanobarcodes©. Our flow-based detection scheme leverages microfluidics and alternate current (AC) electric fields to align and image particles in a well-defined image plane. Using analytical models of the particle rotation in electric fields we can optimize the field strength and frequency necessary to align the particles. This detection platform alleviates loss of information in solution-based assays due to particle clumping during detection.

Keywords: Electrophoresis, Electrorotation, Microparticles

1. INTRODUCTION

Rod-shaped metallic particles are commercially available with 30 nm to 1 μm diameters and lengths of 1 to 10 microns (Nanoplex, Menlo Park, CA). The metallic rods can be electroplated with identifiable stripes that encode on the order of 10 bits of information. Each particle therefore carries a "barcode" that identifies a hybridization reaction or immunoassay that can be performed and detected in parallel with many other particles in a reaction chamber or fluidic channel [1]. The "barcode" pattern of each particle can be identified from the contrast in backscatter intensity of the metal stripes along its length.

Current Nanobarcodes©-based multiplexed assays use bench top sample preparation to bind relevant target and reporter molecules to their surfaces. The particles and sample are then placed in well plate chambers and imaged for analysis using a CCD camera after they settle on the bottom surface of the well [2,3]. A typical example of such images is shown in Figure 1. Particle striping patterns and target binding events are identified in the 2D images using automated image analysis. Assay information from overlapped particles must be discarded, however, to avoid errors. Clumping of particles therefore results in a loss of information during analysis and post-readout particle separation is difficult.

Figure 1: Reflectance-mode image of particles at the surface of a well plate.

2. THEORY

We have developed analytical models for the rotation of rod-like particles with solid metal stripes in DC and low frequency AC electric fields. The model employs the Poisson equation to determine the non-uniform "induced" zeta potential at the particle surface due to polarization of the electric double layer (EDL) [4]. The "native" zeta potential for striped particles also varies along the length of the particle because of the differing zeta potential values for each metal. The zeta potential of the metals is determined from experimental values for homogenous particles. The native and induced zeta potential distributions are superimposed to determine the net potential along the particle surface.

The rotational electrophoretic velocity of the particle is found by solving the Navier-Stokes equations with an additional term to account for the body force on the charge in the EDL [5]. The solution for the rotational velocity of the particle is a function of the angle, θ, between the particle and the applied field and a second order function of the field strength. This velocity was included as a forcing function in the rotational convective-diffusion equation, which was solved analytically to determine the probability density function (PDF) for the angle of a particle with respect to the applied field when Brownian motion is included.

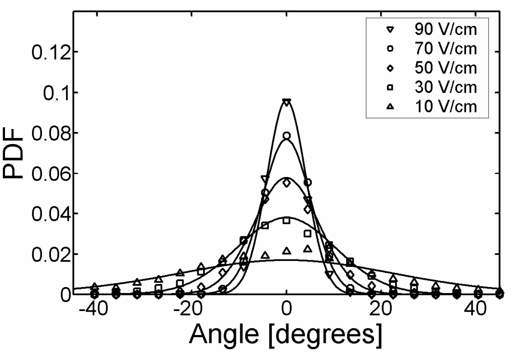

Figure 2: Orientation distributions of half silver/half gold rod-like particles with a relatively thin electric double layer. The symbols are experimental data and the solid lines are model predictions.

To validate our model for particle alignment, we have measured the orientation of particles in a microfluidic device with AC electric fields of order 100 V/cm and ranging in frequency between 50 and 1000 Hz. Our experimental setup uses pattern recognition and particle tracking to simultaneously track the translation, diffusion, and alignment of cylindrical particles 6 microns long and 0.25 microns in diameter under settling conditions and in both DC and AC electric fields. Figure 3 compares model predictions and experimentally observed alignment distributions of particles in 100 Hz AC fields.

3. EXPERIMENTAL

Our flow-based detection scheme, shown in Figure 3, images particles with alternating gold and silver stripes as they flow and settle within a vertically oriented microchannel. The flow cell is interrogated using a microscope objective and images are captured with a CCD camera for automated image analysis. The contrast in backscatter intensity of the metal stripes is optimized by illumination with 450 nm wavelength light.

Six unique barcode patterns were selected for the particles in the detection experiments. The particles were suspended in KCl solution to replicate the anticipated conductivity of assay buffer solutions. We apply a 100 V/cm AC electric field at 100 Hz along the axis of the channel to orient particles parallel to the flow direction and facilitate fast readout of particles.

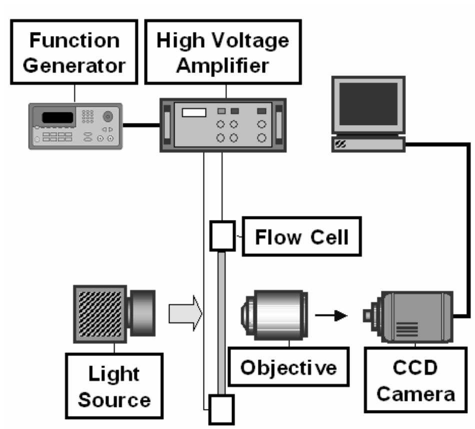

Figure 3: Schematic of the setup for flow based particle control and detection. The flow cell is oriented along the gravitational vector to allow particles to settle without interacting with the sidewalls.

4. RESULTS AND DISCUSSION

Results of the flow-based detection are shown in Figure 4. The striping patterns of particles within the image plane were readily identified from the images. The applied field maintained the particle orientation along the flow direction although Brownian displacements caused the particles to occasionally move in and out of the image plane. Increasing the field strength beyond 100 V/cm or the frequency above 100 Hz did not improve particle detection.

Initial results demonstrate that the striping pattern of particles can be recognized "on the fly", although further improvements to the system are needed to concentrate the particles within the focal plane. The flow-based detection allows integration of the readout with other microfluidic functions such as mixing, buffer exchange, and/or particle separation.

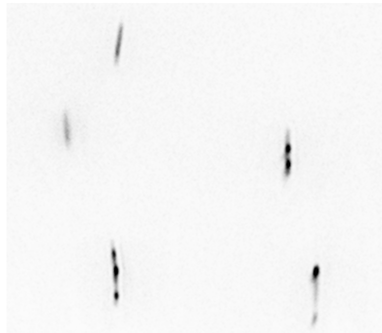

Figure 4. Reflectance-mode image of particles flowing in the vertical microchannel. The particles are aligned in the flow direction with an applied 100 V/cm AC field at 100 Hz.

5. SUMMARY

We have developed a flow-based detection system for optical detection of rod-shaped barcode particles. The system uses AC electric fields to align particles in a vertical flow cell for improved detection. The use of this system can reduce the loss of information due to particle clumping and enable integration of the readout process with on chip sample preparation and separation. The throughput of the flow-based system can be improved to exceed that of a wellplate based system by increasing flow speed and capture rate of the optics.

ACKOWLEDGEMENTS

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