Nanofluidic preconcentration and detection of nanoparticles

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The fast detection and characterization of nanoparticles, such as viruses or environmental pollutants, are important in fields ranging from biosensing to quality control. However, most existing techniques have practical throughput limitations, which significantly limit their applicability to low analyte concentrations. Here, we present an integrated nanofluidic scheme for preconcentration and subsequent detection of nanoparticle samples within a continuous flow-through system. Using a Brownian ratchet mechanism, we increase the nanoparticle concentration ~ 27 -fold. Single nanoparticles are subsequently detected and characterized by optical heterodyne interferometry. A wide range of potential applications can be foreseen, including real-time analysis of clinically relevant virus samples and contamination control of processing fluids used in the semiconductor industry. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4731250>]

I. INTRODUCTION

The rapid and accurate detection and characterization of nanoparticles is of great importance in several fields such as virology, environmental monitoring, and process control in semiconductor manufacturing.¹ In the field of virology, for example, it is critical to accurately quantify virus particles to study the effects of drug therapy in patients; and also to study viral fitness, replication, and inhibition. There are several virus quantification techniques available to virologists, such as the quantitative PCR (polymerase chain reaction) method,² the plaque titer method,³ and the image enhanced microscopy (IEM) technique.⁴ However, a problem common to most of these techniques is that the analysis of a sample involves several tedious steps, which can take several hours to multiple days to complete. To enable quick and accurate characterization of nanoparticle and virus samples, several nanofluidic flow-through schemes have been recently developed.^{5–8} Despite the high speed and accuracy of these techniques, the minimum required concentration is limited to approximately 10^8 particles/ml by the practical throughput capabilities. For the techniques to be applicable for clinically relevant virus samples, they should ideally be able to assess samples with concentrations $\leq 10^6$ particles/ml.⁹ One possible solution to this problem is to enhance the concentration of the samples using an ultracentrifuge before analyzing them. However, this calls for additional processing steps using expensive equipment, which can typically take several hours to complete.

In this article, we present an integrated nanofluidic scheme which is capable of preconcentration and subsequent detection and characterization of nanoparticle and virus samples within a continuous flow-through system. Preconcentration of a sample is achieved using the phenomenon of Brownian ratchets¹⁰ in a flow-cell, while the concentrated particles are detected on a single particle basis within the same flow-cell using a real-time detection scheme based on heterodyne interferometry.⁶ It is demonstrated that the

technique can be used to enrich the concentration of nanoparticles in a sample up to ~ 27 times the original concentration, and then characterize them on a single particle basis.

II. CONCEPT

We consider a spherical nanoparticle moving in a fluidic chamber whose length and height (both > 1 mm) are much greater than its depth (< 500 nm). Fig. 1 shows such a chamber, where we consider motion of the particle in the x - and y -directions. Hydrodynamic effects due to the depth of the chamber are neglected. An external electric field E is applied across the chamber as indicated in Fig. 1, and electro-osmosis is suppressed by adding a reagent (such as POP-6) to the solution. Therefore, the only external force acting on the particle is the electrophoretic force,¹¹ and the particles move in the positive y -direction with a velocity

$$v_y = \mu_e E, \quad (1)$$

where μ_e is the electrophoretic mobility of the particle. Absence of any external force on the particle in the x -direction will allow it to freely diffuse in that direction. In a time step t , the particle will have a diffusion length randomly distributed within a normal distribution of mean 0 and variance $2Dt$. The diffusion length is given by

$$l = \sqrt{2Dt}, \quad (2)$$

where D is the diffusion constant^{12,13} for the motion of a particle in the fluid, given by

$$D = \frac{k_B T}{6\pi\eta r}. \quad (3)$$

In Eq. (3), k_B is the Boltzmann's constant, T is the temperature of the system, η is the dynamic viscosity of the fluid, and r is the radius of the nanoparticle. There will also be a component of diffusion in the y -direction. The diffusion lengths in the y -direction will be randomly distributed with

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the same mean and variance as in the x-direction. As seen in Fig. 1, we consider a periodic array of obstacles within the fluidic chamber, placed throughout the chamber with an x-period x_d and y-period y_d . These obstacles prevent the particles from freely diffusing in the x-direction, and rectify the Brownian motion of the particles such that on an average they move towards one side of the flow-cell. A similar mechanism has been previously used to obtain separation and sorting of DNA molecules of different sizes in continuous flow-through schemes.^{14–18} However, the applicability of Brownian ratchets in concentration enrichment of nanoparticle solutions has not been explored before.

We now evaluate the efficiency of the ratchet layout (in Fig. 1) in the enrichment of sample concentrations, by simulating the motion of a collection introduced into such a fluidic chamber. Fig. 2(a) shows a simulated histogram for a spatially random uniform distribution of 1000 polystyrene nanoparticles across a flow-cell of width $X = 2$ mm. The applied electric field across the flow-cell is $E = 100$ V/cm. Fig. 2(b) is a simulated graph showing the mean and standard deviations of the particle positions within the ratchet. The dimensions of the obstacles and the gaps between them are indicated in Fig. 2(b). The black circles indicate the mean x-positions after every 100 s from when the particles are introduced in the chamber. The graph indicates that the structure shown in Fig. 1 can be used effectively for concentration enrichment of nanoparticles in solution, by moving all particles transversely to one side of the flow-cell and by

FIG. 1. Brownian Ratchet array. Fluidic chamber with an array of periodic obstacles that can act as Brownian Ratchets and rectify the motion of nanoparticles preferentially towards one direction (positive x-direction).

FIG. 2. Simulated demonstration of concentration enrichment in a flow-cell containing a Brownian ratchet array. (a) Random uniform distribution of particles at the top end of an array like that shown in Fig. 1. (b) Means and standard deviations of particle positions in the flow-cell as they travel in the y-direction (downwards in Fig. 1). Each point in the graph is indicated after 100 s of travel within the flow-cell.

confining them along a narrow region. The simulation puts the concentration enrichment at 610, using the ratio of the standard deviations of particle positions at the top and bottom of the array. The particles can then be diverted to a narrow exit channel at the end of the array. We experimentally demonstrate this below.

III. EXPERIMENTS

Fig. 3 shows the schematic and images of the flow-cells that were fabricated for preconcentration and subsequent detection of nanoparticles in a continuous flow-through scheme. The flow-cells were fabricated in fused silica wafers using UV lithography,¹⁹ and were 400 nm deep. The schematic is based on the actual CAD (computer-aided design) used to make the mask containing the ratcheting array and the relatively large features in the flow-cell. Three different regions can be demarcated in the flow-cell—an inlet section for introducing the sample into the flow-cell, a concentrator section, and a detection section. The concentrator section contained an array of obstacles of exactly the dimensions

FIG. 3. Schematic of the fluidic network used for preconcentration of nanoparticles using Brownian ratcheting by obstacle arrays. The unetched areas are shown in red. The reservoirs are marked with yellow circles. Insets (A)-(C) show CCD-acquired optical images of different regions of the flow-cell. (A) A portion of the concentrator section, (B) entrance to the detection section, and (C) nanochannels in the detection section.

used for the simulation shown in Fig. 2(b). Inset (A) of Fig. 3 is a CCD-acquired optical image of the concentrator section showing the array of obstacles in that region. Even though Fig. 2(b) suggests that the mean positions of the particles can be diverted to the right side of the obstacle array (cf., Fig. 1) within a y -travel distance of ~ 5 mm, the length of the ratchet array in the y -direction (x - and y -directions indicated in Fig. 3 for illustration) was made 8 mm. This allowed for slight differences between the performances of the fabricated and the simulated devices. At the end of the ratcheting array, the particles were diverted to a $100\ \mu\text{m}$ wide detection arm containing an array of nanochannels, for the detection of individual nanoparticles using heterodyne interferometry.⁶ These nanochannels were $15\ \mu\text{m}$ long and $400\ \text{nm}$ deep and $500\ \text{nm}$ wide. CCD-acquired images of the entrance to the detection region, and the nanochannels, are shown in insets (C) and (B), respectively. Each flow-cell consisted of three reservoirs for sample application and manipulation (labeled 1, 2, and 3 in Fig. 3). The different structures in a flow-cell were patterned on a bottom wafer, while the reservoirs were fabricated on a top wafer. The two wafers were bonded to obtain sealed flow-cells.

Upon entering the $100\ \mu\text{m}$ wide channel, the particles are quickly redistributed uniformly across the channel, thus lowering the anticipated maximum enrichment of 610 down to approximately 20. The latter enrichment coefficient is determined by the ratio of the widths of the inlet and detection sections of the flow-cell. The width of the detection

section was not made narrower to avoid clogging around the entrance to the detection section due to high aspect ratio between regions inside and outside the detection section.

IV. RESULTS

To evaluate the performance of the flow-cells, a sample of $100\ \text{nm}$ diameter fluorescent polystyrene beads was prepared in $0.5\times$ Tris-Borate-EDTA buffer (50 mM Tris base, 45 mM boric acid, and 0.5 mM EDTA) with 0.1% POP-6 polymer added to it.¹⁶ Fluorescent beads were chosen so that the motion of individual particles inside the flow-cell can be monitored using a CCD camera. The concentration of the sample was determined by transporting the fluid through a flow-cell containing nanochannels of the same dimensions as described above, but without the concentrator section present.⁶ A collimated laser beam was tightly focused using an inverted microscope objective onto the center of a nanochannel. Flow was induced using a pressure-driven mechanism, and the flow-speed was adjusted such that a single particle traversed the laser focus in time $dt \leq 1\ \text{ms}$. The light scattered by the particle while traversing the focus was collected by the same objective and was superimposed with a frequency-shifted reference beam on the surface of a split photodetector. This interferometric signal was demodulated using a lock-in amplifier, hence allowing us to eliminate phase and characterize the particle based on the signal amplitude. The signal amplitude reflects the particle's

FIG. 4. Real-time traces and size distributions of detected nanoparticles. (a) Real-time trace (4 s) of the detector signal obtained during analysis of a polystyrene bead sample before concentration enrichment. (b) Detector time trace obtained in the detection section of a flow-cell with a preconcentrator. Note that the particle counts are significantly higher in (b), indicating a concentration enrichment. (c) Typical histogram obtained for the size distribution of particles detected in the samples. The cumulative particle count is used in determining the sample concentration.

polarizability, and provides information about the particle's size. For an ensemble of particles traversing the laser focus, a histogram can be generated for the size distribution of the

particles present in the sample. We have previously shown that this method based on heterodyne interferometry can be used to characterize nanoparticles and viruses with unsurpassed sensitivity, accuracy, and size resolution.^{6,7} In addition to size-related information, the concentration of the sample can also be determined. Since the time taken by a particle to traverse the laser focus dt is known from the time-dependent detector output, and the size of the laser focus dx is defined by the NA of the objective, the particle velocity (and hence the fluid velocity) dx/dt can be determined. Using the known cross-section A of a nanochannel, the volume flow-rate of the sample $dV/dt = A dx/dt$ can be determined. For n particles detected in time τ , the concentration of the sample is the ratio $\frac{n}{V}$, where $V = \tau dV/dt$ is the total volume of sample analyzed. Using this analysis procedure, the concentration of the polystyrene bead sample was determined to be 5.2×10^{10} particles/ml. Fig. 4(a) shows a time-trace of the detector signal obtained when the sample was analyzed.

The above sample was used to determine the efficiency of several preconcentration flow-cells (i.e., flow-cells with a concentrator section). A typical experiment proceeded as follows. $2 \mu\text{l}$ of the sample was introduced in reservoirs 1 and 2 of a preconcentrator flow-cell, and a 100 V/cm electric field was applied across the reservoirs in the direction shown in Fig. 3. The electric field was applied for 17 min, after which it was stopped and a vacuum was created in the detection section from reservoir 3, hence inducing pressure-driven flow of particles in this section. The concentration of particles being detected in a nanochannel in this section was then evaluated in the same way as for the original sample before concentration enrichment. Fig. 4(b) shows a time-trace of the photodetector signal obtained in the detection section of a preconcentration flow-cell which provided a $26\times$ concentration enrichment. A comparison with Fig. 4(a) clearly shows the significant concentration enrichment attained. Fig. 4(c) shows a typical size distribution obtained by counting individual particles in the detection region of a flow-cell, and calibrating the signal amplitudes to particle sizes.⁶ Table I summarizes the results obtained using 5 different preconcentration flow-cells and demonstrates the actual performance variability among different channels on the same wafer. The concentration enrichment factors were found to be in the range $15\times$ to $27\times$.

Note that in determining the sample concentrations (cf., Table I), the average particle flow speeds through the focus are determined. There is a $\sim 25\%$ variation in particle flow-speeds during the data acquisition time-frame, which produces a systematic error of $\sim \pm 25\%$ in the determination of sample concentrations.

TABLE I. Comparison of sample concentrations determined before and after enrichment with different preconcentration flow-cells.

Initial concentration	Enriched concentration	Enrichment factor
5.2×10^{10}	7.8×10^{11}	15X
5.2×10^{10}	9.7×10^{11}	18X
5.2×10^{10}	13.9×10^{11}	27X
5.2×10^{10}	13.7×10^{11}	26X
5.2×10^{10}	10.3×10^{11}	20X

V. CONCLUSIONS

The integrated nanoparticle preconcentration and detection scheme presented above can potentially find applications in various fields where fast and accurate analysis is required for samples with low analyte concentrations. The enrichment factors obtainable with the above scheme could be further enhanced by reducing the cross-section of the detection section of the flow-cell (cf., Fig. 3), while avoiding the risk of particle clogging. With such improvements, this scheme could be implemented for rapid and efficient analysis of clinically relevant virus samples, thus eliminating the need for intermediate processing steps using expensive equipment.

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