ACOUSTIC TRAPPING EFFICIENCY OF NANOPARTICLES AND BACTERIA

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ABSTRACT

In this paper we present a method to characterize the acoustic trapping efficiency of nanoparticles and bacteria when using seeding particles. Through the use of fluorescent microscopy and video analysis, single particles/bacteria were counted as they entered the acoustic trap at different flow focusing ratios and by comparing the amount of trapped objects to the amount of objects that were lost from the trap, the trapping efficiency could be calculated. Using fluorescent 780 nm polystyrene particles, an optimization of the hydrodynamic sample pre-focusing could be performed. For a flow focusing ratio of 1:10 or 5:6 (sample:sheath flow), a trapping efficiency of around 90% could be achieved at a total flow rate of 11 µl/min. At a flow focusing ratio of 1:10, GFP-producing *E. coli* could be trapped at an efficiency of above 95%. Using this characterization technique, important aspects of the acoustic trapping method (e.g. transducer frequency and voltage, size and type of seeding particle, amount of flow focusing, total flow rate etc.) can be characterized and optimized.

KEYWORDS

acoustic trapping, trapping efficiency, nanoparticles, bacteria.

INTRODUCTION

Acoustic trapping has the potential to become an important tool for enrichment and washing of dilute samples in microfluidic systems. The introduction of seeding particles in acoustic trapping, as first presented at µTAS 2011[1], enabled trapping of particles that were previously thought too small to manipulate. This opened up for applications aimed at pathogenic bacteria enrichment, e.g. bacteremia diagnostics. However, considering the low numbers of bacteria present in clinical samples (often <1 cfu/ml), it is paramount to trap as many bacteria as possible if a subsequent detection is to be possible. We here present a new method for characterizing the acoustic trapping efficiency of nanoparticles and bacteria and demonstrate an optimization of the hydrodynamic sample pre-focusing.

EXPERIMENT

The trapping platform consists of a rectangular borosilicate capillary (2 x 0.2 mm^2) with a sheath flow inlet and a sample flow inlet, see figure 1. The capillary is clamped to a printed circuit board (PCB). On the PCB, a diced piezoelectric transducer (0.9 x 0.9 mm^2) with a resonance frequency at 4 MHz is mounted. When activating the transducer, a local standing wave is created in the capillary above the transducer and when particles are transported to the trapping zone they will be retained in the pressure node. Good acoustic coupling between the transducer and the capillary is ensured by a thin layer of glycerol. Further details of the trapping platform can be found elsewhere[2]. In order to perform the trapping efficiency measurements, two modifications of the previously presented platform were implemented: A 100 nm thick layer of chromium was deposited on the backside of the capillary to decrease background fluorescence from the PCB and a sample inlet was added to the top of the capillary to enable hydrodynamic sample pre-focusing.

At the start of each measurement, 12 µm polystyrene beads were aspirated through the capillary outlet and trapped to act as seeding particles, as shown in figure 2. 780 nm fluorescent polystyrene particles were then infused through the sample inlet and hydrodynamically focused using deionized water as sheath flow. The flow ratio of the sample and sheath flow was either 1:10, 5:6, 10:1 or 11:0 with a fixed total flow rate of 11 µl/min. The particle concentration was sufficiently low to ensure single trapping events. For the measurement with sample flow at 1 µl/min and sheath flow at 10 µl/min $1 \cdot 10^5$ particles/ml were used and for all other flow settings $1 \cdot 10^4$ particles/ml were used. For bacteria measurements, GFP-producing *E. coli* XL-1 blue ($5 \cdot 10^4$ bacteria/ml) were used.

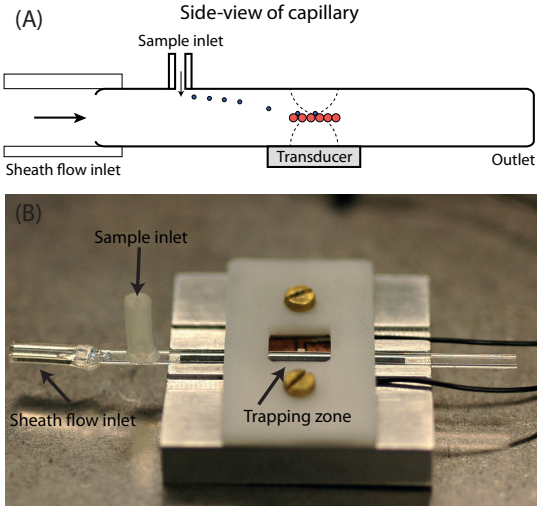

Figure 1. A 4 MHz piezoelectric transducer creates a standing wave with a pressure node in the center of the capillary where both seeding particles and nanoparticles are trapped against the flow (A). The capillary is clamped on a printed circuit board (PCB) using an aluminum/plastic fixture (B).

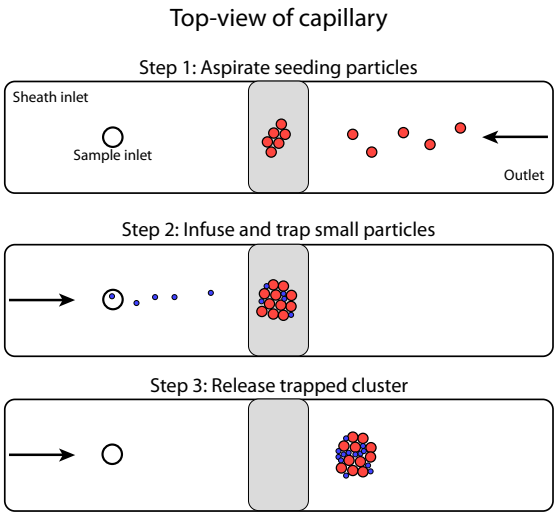

Figure 2. The capillary has two inlets (sheath fluid and sample) and one open outlet. In step 1, seeding particles are aspirated through the outlet and trapped over the transducer. In step 2, nanoparticles or bacteria are infused through the sample inlet, hydrodynamically focused with different flow ratios and trapped in the seeding cluster. In step 3, the ultrasound is turned off and the trapped cluster is released.

The trapping efficiency (percentage of trapped particles) was measured by counting the particles/bacteria entering and exiting the trap. High-speed images were acquired using a CCD and analyzed in MATLAB®. For each measurement, a region-of-interest (ROI) that all particles passed through when entering the seeding cluster was determined. Within this ROI, the mean fluorescent intensity of each pixel row was calculated in all video frames to capture the increase in fluorescence intensity that a passing particle/bacteria would create, see figure 3. The resulting array of intensity peaks was filtered using a Savitzky-Golay filter to remove any background variations and finally a threshold level was set that enabled the counting of any individual particles or bacteria that passed through the ROI.

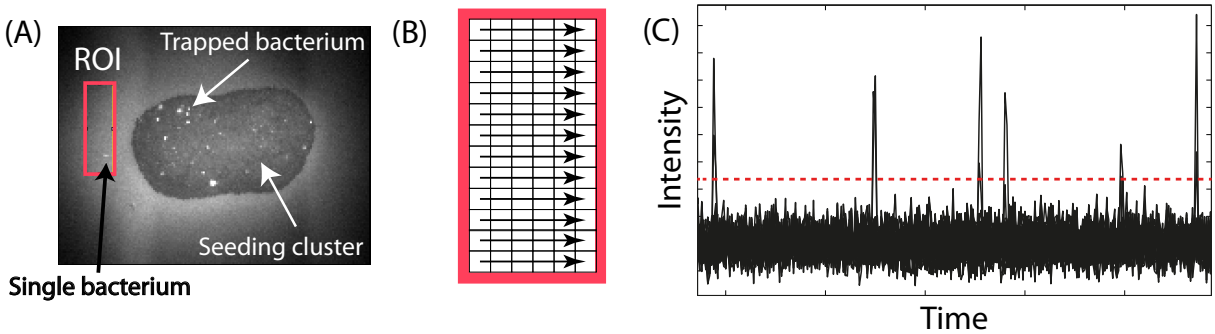

Figure 3. (A) shows a video frame from the bacteria trapping experiment. Using the hydrodynamic sample focusing the ROI could be selected so that all bacteria entering the cluster goes through it. Trapped bacteria can be seen as bright spots in the grey cluster of seeding particles. The average of each pixel row in the ROI is then calculated for each video frame (B) and the result is a timeline with clear fluorescent peaks for each single bacterium passing the ROI (C). The dashed red line is the threshold for peak detection.

RESULTS

The presented method to characterize the acoustic trapping efficiency was successfully applied to nanoparticles and bacteria. Using 780 nm polystyrene particles and a hydrodynamic sample pre-focusing ration of 1:10 or 5:6, a trapping efficiency of around 90% was achieved. A sample pre-focusing ratio of 10:1 or using no focusing, 11:0, resulted in a trapping efficiency of around 55%, see figure 4. A 1:10 pre-focusing ratio results in a slightly higher trapping efficiency, but the 5:6 pre-focusing ratio enables a sample infusion rate that is five times greater while almost maintaining the same trapping efficiency.

GFP-producing *E. coli* were also tested to see if their trapping behavior would differ significantly from the nanoparticles that were used for the pre-focusing characterization. Using a pre-focusing ratio of 1:10, a trapping efficiency of 96.9 ± 2.8% was achieved, see figure 5.

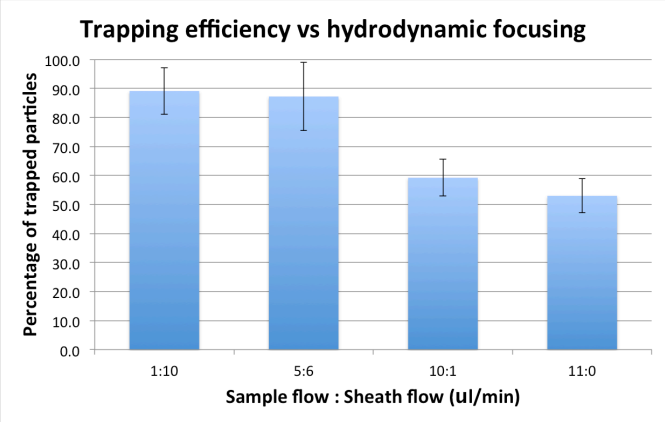

Figure 4. Trapping efficiency of 780 nm polystyrene particles versus hydrodynamic focusing. Error bars show ± 1 standard deviation and n = 3.

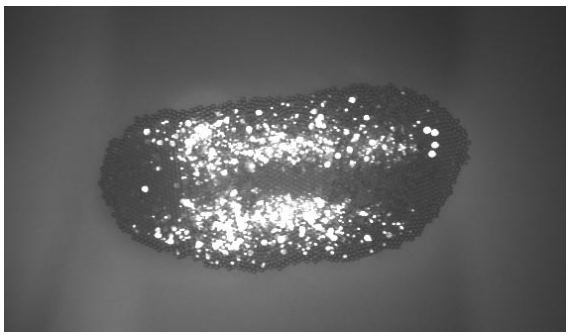

Figure 5. An image of a cluster of 12 µm seeding particles. Trapped GFP-producing E. coli can be seen as bright spots between the seeding particles. The bacteria are trapped in a pattern that is dependent on the acoustic streaming in the system.

CONCLUSION

The results show that high trapping efficiencies can be achieved when enriching bacteria-sized objects using hydrodynamic sample pre-focusing. The new characterization method enabled optimization of the sample pre-focusing and would also enable optimization of other parameters affecting the trapping performance, e.g. total flow-rate, seed particle size and material and the acoustic actuation.

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