

# LOW ASPECT RATIO RESISTIVE PULSE SENSOR FOR SINGLE CELL ANALYSIS

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## ABSTRACT

This paper reports the use of low-aspect ratio micropores for analysis of inorganic particles and bacterial cells using resistive pulse technique. 5  $\mu\text{m}$  diameter pores were drilled in 50 nm thick free standing silicon nitride ( $\text{Si}_x\text{N}_y$ ) membranes to obtain an aspect ratio of 0.01. Low-aspect ratio pore results in high spatial resolution along the analyte length as the ionic current blockade corresponds to sub-parts of analyte present inside the pore. In our experiments, population statistics and shapes of the current blockades were used to determine size and polydispersity for microparticles and distinguish flagellated from non-flagellated bacteria.

**KEYWORDS:** Resistive Pulse Sensor, Micropore, Single-cell detection

## INTRODUCTION

When analyte particles suspended in an electrolyte solution are electrophoretically driven across a micropore (made in an insulating material), they displace electrolyte solution from the pore volume and transiently increase the electrical resistance of the pore. These changes in pore resistance are termed as 'resistive pulses' and their magnitude, shape and frequency can be used to obtain information about the size distribution and the concentration of particles /cells in the solution. This principle has been used over the decades in Coulter counters [1] for cell counting; however, the sensing pores in conventional Coulter counters and their miniaturized MEMS versions [2, 3] are typically several micrometers long, and fail to provide reliable shape information for the analyte. They also often result in false positive signals when two or more particles simultaneously enter the pore or when particle aggregates are present. These shortcomings of resistive pulse sensors can be addressed by improving the sensing resolution by decreasing the length of the micropore. In this study, we use micropore with pore length of only 50 nm for high resolution sensing of microparticles and bacterial cells. When using such thin pores,  $\text{Diameter}_{\text{particle}} \gg \text{Length}_{\text{pore}}$  and at any instant only a fraction of the translocating particle is present inside the pore, enabling us to obtain reliable size and shape information.

## EXPERIMENTAL

For micropore chip fabrication, 50 nm thick  $\text{Si}_x\text{N}_y$  layer was deposited on a silicon wafer on either side. A 50  $\mu\text{m}$  x 50  $\mu\text{m}$  window was opened in silicon wafer using photolithography and standard wet etching to obtain 50 nm thick free standing  $\text{Si}_x\text{N}_y$  membrane [4, 5]. A solitary 5  $\mu\text{m}$  pore (Figure 1(b)) was drilled using focused ion beam (FEI Strata DB 235). Figure 1(c) shows the schematic of final micropore chip. The chip was cleaned and assembled in the flow cells as shown in Figure 1(a), flow cell was filled with 50 mM potassium chloride (KCl) and transmembrane voltage of 50 mV was applied using Molecular Devices Axopatch 200B. The polystyrene beads (1, 2 and 3  $\mu\text{m}$  beads, Polysciences, Inc. Warrington, PA) were diluted in the same electrolyte solution to a final concentration of  $10^5$ - $10^6$  particles/ ml. The bacterial cells (*Salmonella typhimurium*) were grown overnight in LB broth and were washed in phosphate buffer 2-3 times by mild centrifugation and diluted to similar final concentration in KCl. The analyte were introduced in the upper chamber of the flow cell and soon resistive pulses corresponding to translocation events were observed. Each pulse was characterized by ionic current drop magnitude and pulse width which correspond to diameter of the translocating particle and its translocation duration.

## RESULTS and DISCUSSION

The translocation of particles and cells through the micropore resulted in current drop events with high signal-to-noise ratio. The current-time traces were recorded and event magnitude and time were

extracted using thresholding method in Clampfit ver. 10.3.1.4 (Molecular Devices). Figure 1 (d) shows the current drop histograms for 1, 2 and 3  $\mu\text{m}$  particles and the inset in Figure 1 (d) shows the current drop events for the three particle sizes. Based on the histograms, 1 and 2  $\mu\text{m}$  particles show tight normal distribution, however, current drop distribution for 3  $\mu\text{m}$  particles has two peaks suggesting high polydispersity index for these particles. Moreover, the histograms for all three particles are well separated demonstrating the ability of our device to distinguish between different size particles.

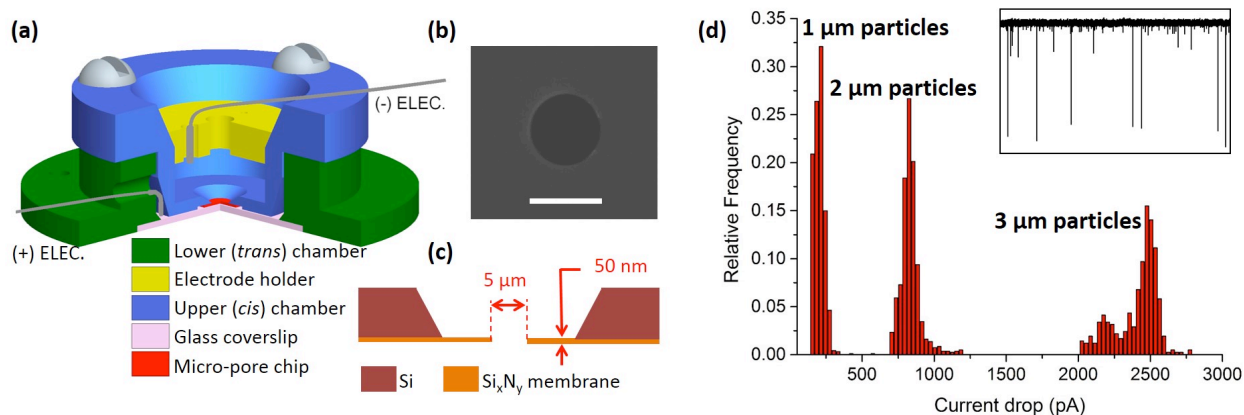


Figure 1: (a) Flow-cell set-up. Ag/AgCl electrodes are to either electrolyte chamber for voltage clamping. (b) SEM image of the drilled pore. Scale bar: 5  $\mu\text{m}$ . (c) Schematic of the micropore chip. (d) Histograms for polystyrene particles translocation events. Insert shows typical resistive pulses for the three particle sizes.

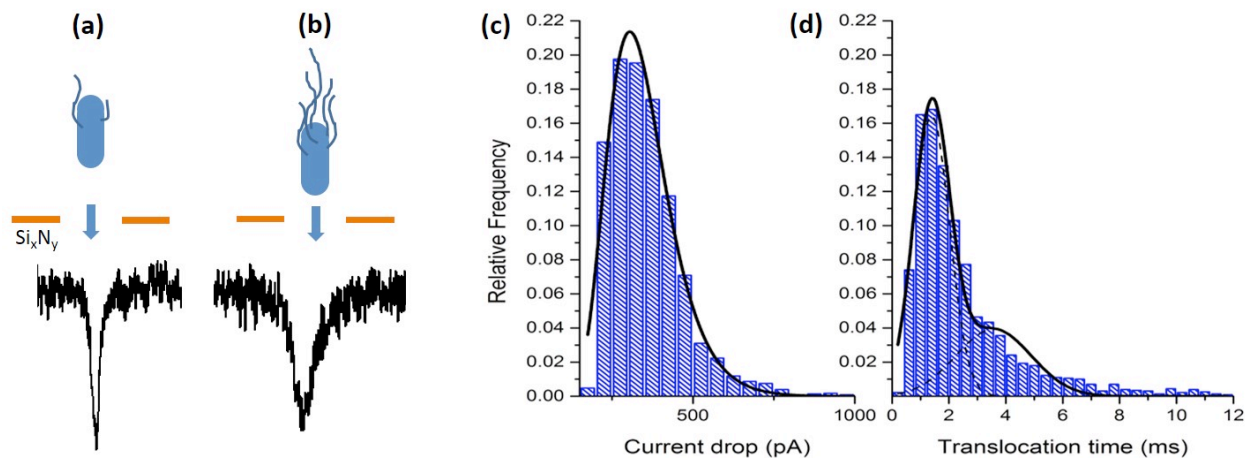


Figure 2: Current shape for (a) non-flagellated and (b) flagellated bacterial translocation. Non-flagellated bacteria appear in the sample during washing and centrifugation. (c) Current drop histogram for bacterial translocation events. A current drop level similar to that for 1  $\mu\text{m}$  particle indicates bacterial translocation in orientations depicted in (a) and (b). (d) Translocation time histogram. Two peaks correspond to non-flagellated and flagellated bacteria.

For bacterial translocation, we observed events with highly variable translocation duration. *S. typhimurium* are flagellated bacteria with multiple flagella on the cells surface, however, they tend to shed off the flagella during centrifugation. We attributed the variability in translocation duration to the presence of heterogeneous bacterial population with and without flagella. Figure 2 (a) & (b) shows different pulse shapes obtained for bacterial translocations along with the possible cell orientations. Figure 2 (c) shows current drop histogram for the translocation events. The histogram was fitted with a

log-normal curve to obtain the most probable current drop value and a peak value of 332.46 pA was obtained (N=2284). Population statistics for current drop values suggested that bacteria translocate in longitudinal orientation as depicted in Figure 2 (a) & (b) as the current drop values were similar to those for 1  $\mu$ m particles. Figure 2 (d) shows the histogram for translocation duration for bacteria. The histogram had two peaks at 1.38 ms and 3.56 ms corresponding to the two populations of bacteria. We believe the presence or absence of flagella changes the protein distribution on bacterial cell body which in turn affects its electrophoretic behavior. Since, the flagellated and non-flagellated bacteria would have different electrophoretic mobility values; we see two peaks in translocation time distribution.

## CONCLUSION

We have demonstrated the use of a low-aspect ratio resistive pulse sensor for high throughput single microparticle and single cell sensing. Our sensor set-up provides high resolution size and shape information and may be applied to distinguish amongst cell populations with different shape parameters.

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