Periodic Signals From a Nanopore Coulter Counter

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***Abstract* – In this work, we report the observation of periodic signals from a Coulter counter that employed glass pipettes. These periodic signals occurred even when no nanoparticles were presented in the counter. Observed phenomenon may be related to the translocation events through glass pipette. Further studies are needed to better understand these results.**

***Index Terms* – Coulter counter, nanopipette, nanopore, translocation.**

I. INTRODUCTION

Traditionally, determination of the size and concentration of nanoparticles has been performed through chromatography, gel electrophoresis, or dynamic light scattering. However the Coulter Counter technique [1] also provides a promising and reliable method for particle counting and sensing in a simpler manner. This method can be briefly described as two chambers, filled with particle-laden solution, separated by a membrane with a single tiny pore. The ionic current through the pore, created by electric potential applied between the chambers, depends on the diameter of the pore, and changes when pore is partially blocked. In most cases the blocking of the pore is caused by translocation of small particles. By monitoring these signals we can count the number of particles translocated through the pore from one chamber to another, and the particle size can be determined, if the pore size is known. This technique is a useful tool for nanotechnology and biomedical applications.

Here we report the observation of periodic signals from certain experiments when nanoparticles were not presented in the chamber. These results are very surprising and counter-intuitive. Here we present main results of our findings and some discussions.

II. EXPERIMENTAL

Nanopipets were fabricated from borosilicate capillaries with inner diameter 0.8 mm and outer diameter 1.5 mm. These capillaries are pulled using a glass pipettes puller (P2000, Sutter, Novato, CA), to achieve an orifice size of a few hundred nanometers. Multiple parameters can be used to control the size of the capillary tip, such as filament current, heating duration, and pulling force.

SEM image of the capillary tips are taken to determine the size (Figures 1 and 2). The nanopipette was filled with 0.1 mol/L potassium chloride (KCl) solution (Fisher Scientific) with pH = 5.5 and submerged in the bath with the same solution. A Ag/AgCl 0.2 mm thick electrode was embedded into the capillary until it reaches the conical part. The reference Ag/AgCl electrode was immersed directly into the bath, as shown in Figure 3. Average distance between electrodes was 5-7 mm. For testing purpose, we check *I-V* dependence, and as expected, the *I-V* curve behaves ohmically. For ionic current recording we used the Axopatch 200B amplifier in voltage clamp mode with a low-pass Bessel filter at 2 or 5 kHz bandwidth. The signal was digitized by Axon Instruments Digidata 1440A Series with sampling rate 250 kHz, and recorded with AxoScope 10.2 (Axon Instruments).

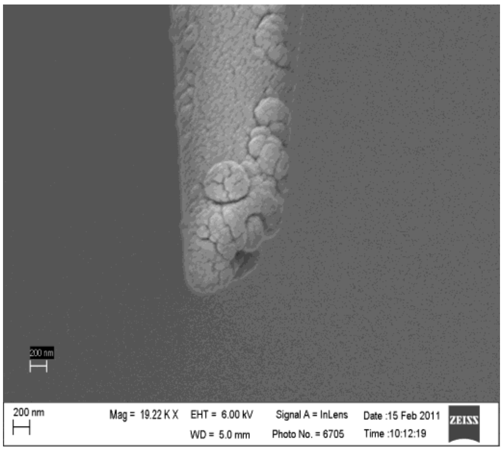

Fig. 1 SEM Image of the nanopipette tip (coated with Pt for SEM)

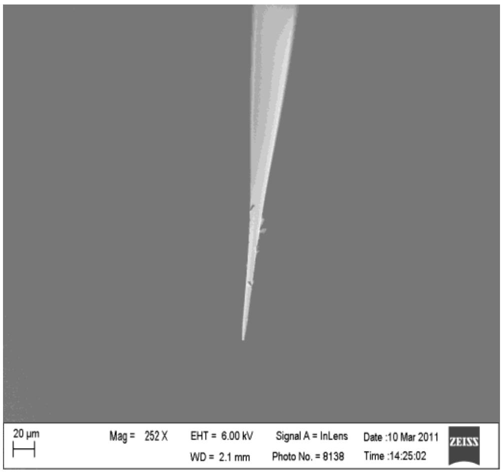

Fig. 2. SEM image of the tips, demonstrating a cone-shaped tip

III. RESULTS AND DISCUSSIONS

The experimental set up is commonly used in Coulter counting of nanoparticles. At usual cases, we observed the typical baseline current and translocation signals, as shown in Figure 4 below.

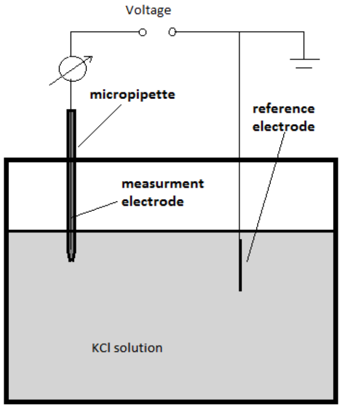

Fig. 3. The schematic set up of our translocation experiment.

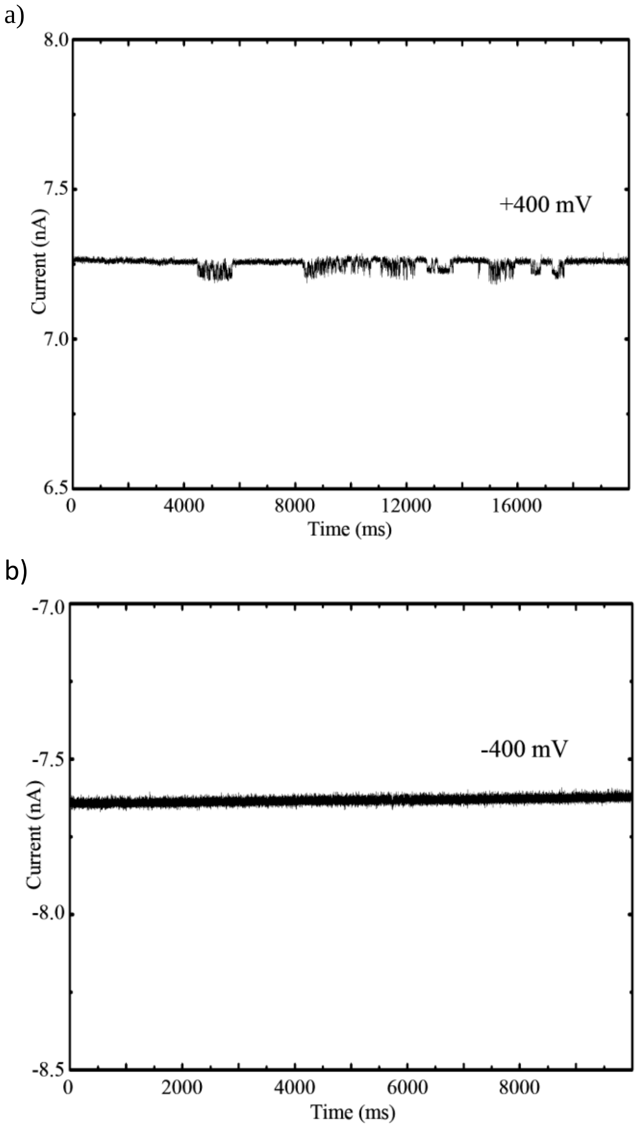

Fig. 4. Particles translocation experiment with pipette of 800 nm pore size, a) shows clear blockade signals with positive +0.4V potential applied, and b) no translocations observed after switching voltage to negative sign.

These signals related to the translocation of nanoparticles, which are blocking the ionic conduction during translocation. As we can see from above, the nanoparticle translocation signal, distributed randomly, are obtained only when positive voltage is applied. While only a baseline current is observed when applying negative voltage. However, we encountered some interesting phenomena when using a syringe to inject the KCl solution into the micropipette. We test it simply applying positive and negative voltage. Without any introduction of nanoparticles, instead of a typical baseline of current that represents the ionic conduction through micropipette, we observed a clear periodic signal shown in Figure 5. This result exhibits a base line current of about 2.51 ± 0.03 nA, which represents the ionic current passing through the micropipette when there is no nanoparticles in the solution. The amount of this current can be estimated by the following equation [2]:

$$I_0 = n(t)\times e \times A(x,t)\times v(x)_{ion} \qquad (1)$$

where $n(t)=n_{+}+n_{-}$ is a sum of positive and negative ion densities, e is elementary charge, $A(x,t)$ is the cross section, $v(x)$ is average ion velocity which is derived from $v(x)$ $=\chi E$, where χ is mobility of K^+ and Cl^- and E is electric field at the orifice.

The multiple dips shown in Figure 5 stand for the sudden drop of current every time the ionic path in capillary is blocked. This change of current is related to the variation of resistance. Figure 6 shows the event current as a function of time using a different micropipette and with an applied voltage of 500mV.

We have conducted further investigation to study this interesting signal which appears in our measurements at room-temperature. We repeated our experiments several times without introducing any nanoparticles; the periodic signals persisted. The periodic signals, which stand for the event current, are uniformly distributed, compare to the randomly pikes in normal nanoparticle translocation picture (Figure 4).

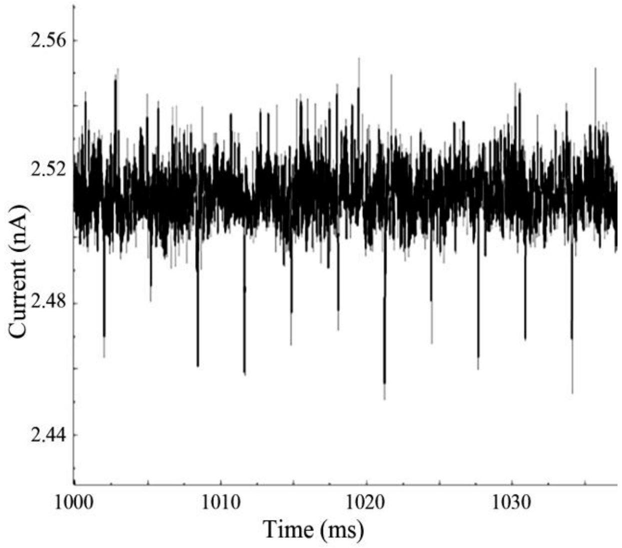

Fig. 5. Event current as a function of time with an applied voltage of 300mV.

As we increase the applied voltage, we notice the frequency of the periodic signals also increases. We found a linear dependence of this frequency on the applied voltage which is not shown here. Interestingly, unlike the translocation signals in Figure 4, these periodic signals reverse their polarity when voltage polarity is switched. The data in Figures 7 and 8 show that when the applied voltage changes to negative voltage the event currents also reverses it direction as expected. However both the frequency of the periodic signals and the magnitude of the event current remain unchanged.

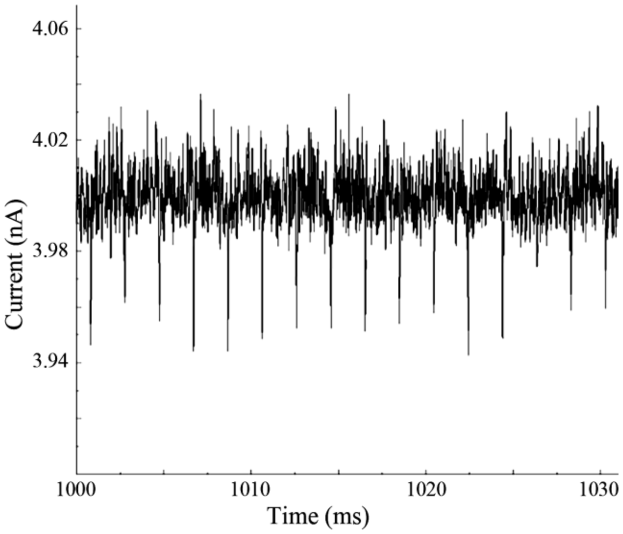

Fig. 6. Event current as a function of time with an applied voltage of 500mV.

The interesting results we observed are hard to explain, since no particles are involved. Extra care and multiple repeated experiments should minimize any possible contamination of nanoparticles in the solution or pipette. In addition, the major challenging fact is the perfect regularity of these periodic signals, which are linearly proportional to the applied voltage. At this moment, we do not have any plausible explanations for our observations. We point that nanobubble in aqueous solution may relate to our observations [3-6].

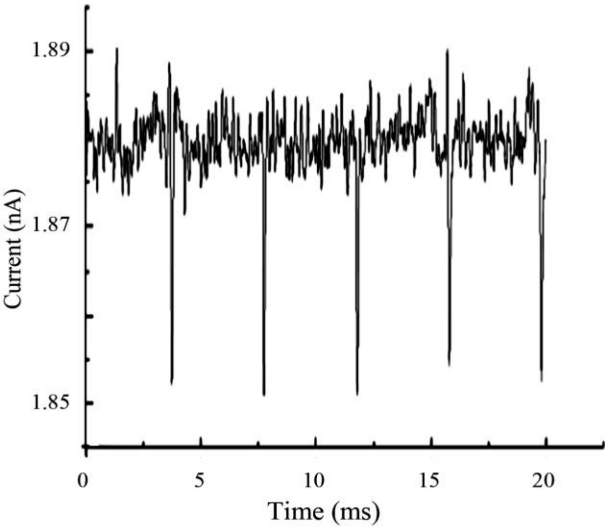

Fig. 7. Signals recorded before switching polarity

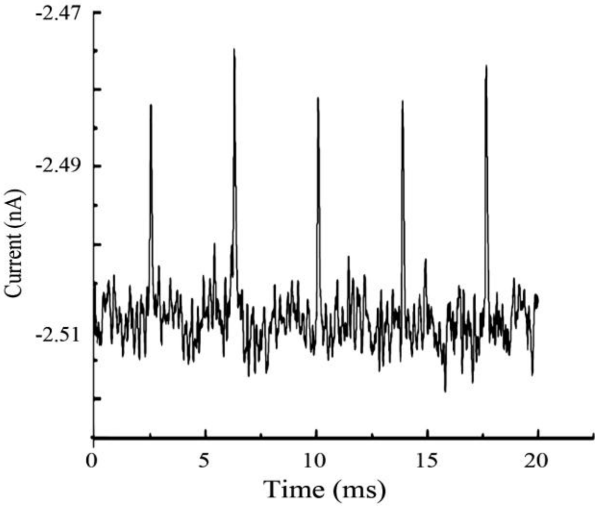

Fig. 8. Signals recorded after switching polarity

IV. CONCLUSION

It has been observed that periodic signals from a Coulter counter that employed micropipette. These signals appeared when a syringe is used to inject KCl solution into the micropipette. The frequency of the periodic signals is linear proportional to the applied voltage. These periodic current signals are reversible under polarity change of the applied voltage.

ACKNOWLEDGMENTS

L. Chow would like to thank National Science Foundation (NSF-ECCS-0901361) for financial support.

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APPENDIX A

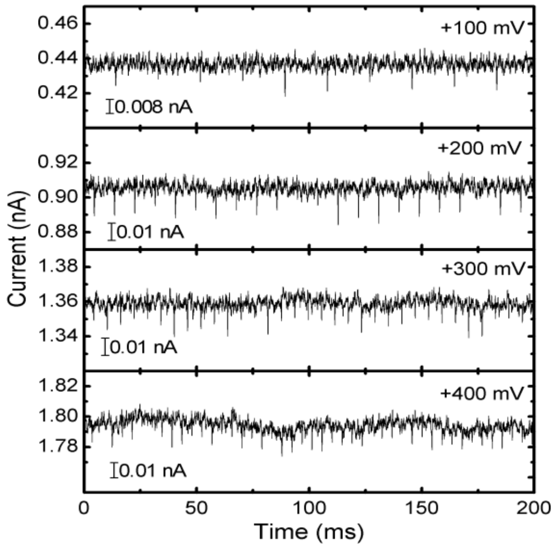

Fig. A. Data recorded in experiment with 200 nm pore size, with different voltages, 100-400 mV.

APPENDIX B

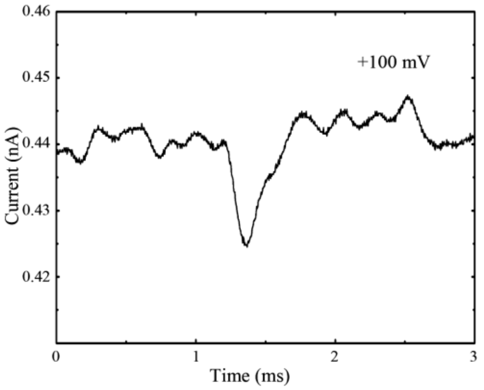

Fig. B. A single peak of data recorded for 200 nm pore size with potential +100 mV

APPENDIX C

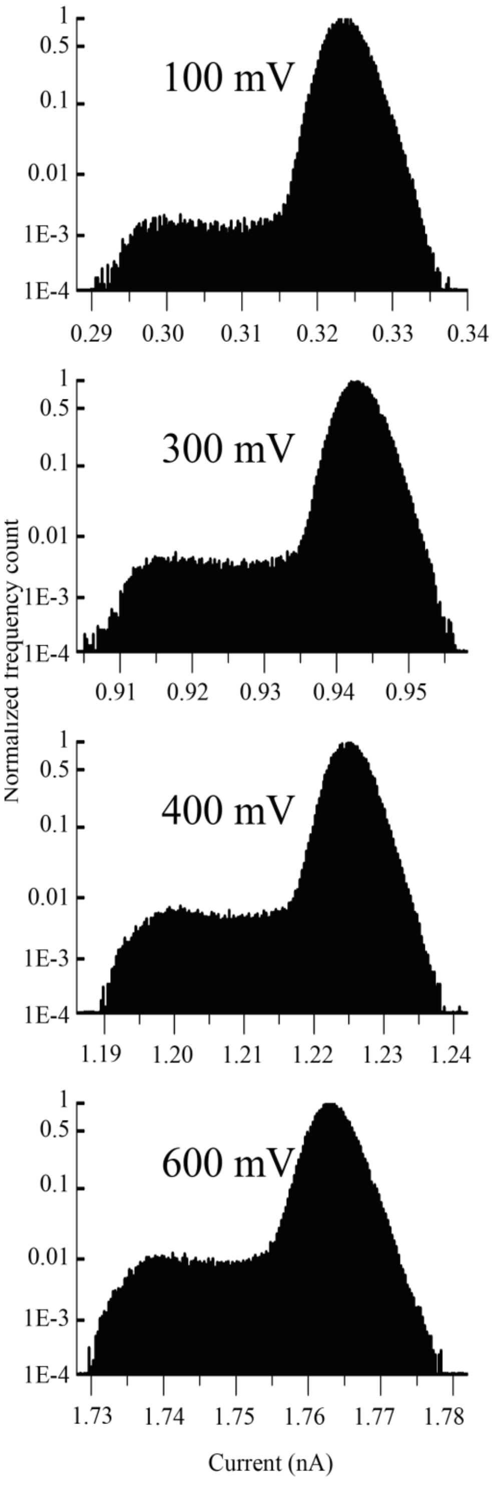

Fig.C. Current histogram of data recorded in a experiment with different voltages. Time of recording is 10 seconds for each voltage. The highest peak indicates a baseline current, when a plateau on the side illustrates the event current. It is also clear from this histogram that frequency of event current is increasing with voltage.