FALSE NEGATIVE AND FALSE POSITIVE FREE NANOPORE FABRICATION VIA ADAPTIVE LEARNING OF THE CONTROLLED DIELECTRIC BREAKDOWN

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ABSTRACT
We investigate the current transport characteristics in the electrolyte-dielectric-electrolyte structure commonly used in the in-situ controlled breakdown (CBD) fabrication of solid-state nanopores. It is found that the stochastic breakdown process could lead to fidelity issues of false positives (an incorrect indication of a true nanopore formation) and false negatives (inability to detect initial nanopore formation). Robust and deterministic detection of initial physical breakdown to alleviate false positives and false negatives is critical for precise nanopore size control. To this end, we report a high fidelity moving Z-Score method based CBD fabrication of solid-state nanopore. We demonstrate 100% success rate of realizing the initial nanopore conductance of 3±1 nS (corresponds to the size of 1.7±0.6 nm) regardless of the dielectric membrane characteristics. Our study also elucidates the Joule heating is the dominant mechanism for electric field-based nanopore enlargement. Single DNA molecule sensing using nanopores fabricated by this method was successfully demonstrated. We anticipate the moving Z-Score based CBD method could enable broader access to the solid state nanopore-based single molecule analysis.

KEYWORDS
Solid-state nanopore, controlled breakdown, moving Z-Score, DNA sensing.

INTRODUCTION
Due to their mechanical robustness, tunable size, and integration potential, solid-state nanopores emerge as promising label-free sensors for detection of single biomolecules. The ionic current blockades during the molecule translocation could provide rich information about the molecule properties. While conventional transmission electron microscopy and focused ion beam achieved considerable success for solid-state nanopore research, their accessibility is limited. Controlled breakdown CBD) method for nanopore fabrication was proposed as an alternative [1]. In this approach, a strong electric field causes a local material failure and results in a nanoscale pore.

Constant voltage stress (CVS) [1] and multilevel pulse-voltage injection (MPVI) [2] are two primary CBD approaches. The pilot work showed a sub-2 nm nanopore could be created by applying a constant voltage across the membrane until a time-dependent dielectric breakdown event occurs. The nanopore formation is signified by the measured membrane current reaching a predetermined cut-off level. Although, the CVS approach could have several reliability issues. The measured current under CVS is the sum of leakage current and the intrinsic nanopore current (if nanopore is formed). The latter can be overwhelmed by the former at high voltages. In addition, the leakage current varies from the membrane to membrane due to the heterogeneous defect profiles. As a result, a universal predetermined cutoff current is impossible to implement. False positive (negative) nanopore formation will occur if the cutoff current is set too low (high). On the other hand, in the MPVI method, alternating high and low voltages are used to stress the membrane and to measure the intrinsic nanopore current, respectively. The repetitive voltage switching creates two fidelity issues due to the capacitive nature of the membrane. The first is the actual voltage across the membrane may not reach the target level if RC time is larger than the high voltage duration. The second is the low voltage measurement after each high voltage pulse must wait until transient current diminishes. Since the RC time is usually unknown and could only be guessed for a specific experiment, long waiting time would be preferred to avoid interfering nanopore current with the transient current. This repeated long waiting time could lead to the total experimental time to be over hours and thus limits the fabrication throughput.

In this work, we demonstrated a consistent and robust CBD nanopore fabrication using a moving Z-Score based adaptive learning approach. This adaptive learning approach monitors the anomalous current event in real time to avoid false negatives. The anomalous event is further examined by IV scanning at low voltages to verify the linearity and conductance consistency to avoid false positives and to reduce the total experimental time. In addition, we also studied the electric field-based nanopore enlargement dynamics. Successful DNA translocation experiments confirm the reliability and precision of the fabricated nanopore by the moving Z-Score approach.

METHODS
Materials and Chemicals
Low-stress SiN_x membranes on 200 μm thick lightly doped silicon substrate were used in our experiments (Norcada, Canada). Ag/AgCl electrodes were house-made with 0.375 mm Ag wires (Warner Instruments). λ-DNA (48.5 kbp, 0.3 μg/μl) was purchased from ThermoFisher. PBS, KCl and Tris-buffer solution (pH 8.0) were purchased from Sigma-Aldrich. Ecoflex-5 used as an insulating sealant of the membrane was obtained from Smooth-On, Inc. Prior to use, all solutions were filtered with a 0.2 μm Anotop filter (Whatman).

Automated moving Z-Scored based nanopore fabrication
The SiN_x membrane was sealed onto a custom-built PMMA flow cell with the *cis* and *trans* chamber. Each side of the membrane was wetted with 5 μl of IPA (isopropyl alcohol) before filling both chambers with 1 M

KCl in 1× PBS buffer. Two Ag/AgCl electrodes were inserted into the KCl solution and electrically connected to a source meter unit (Keithley 2636). The entire assembly is shielded inside a Faraday cage to minimize electromagnetic interferences. A custom-built LabVIEW program automatically samples the total cis-to-trans current at 50 Hz under a constant stress voltage.

DNA sensing

The nanopore was in-situ fabricated with 1 M KCl in 10 mM Tris at pH 8 before the single DNA sensing experiment. λ-DNA was added to the *cis* chamber to a final concentration of 10 μg/ml. The BNC-interfaced Ag/AgCl electrodes were then connected to the Axopatch 200B amplifier with *cis* chamber held at the ground. The amplified signal was filtered with a 4-pole Bessel set at 10 kHz and digitalized by a 16-bit/100 MHz DAQ card. Data analysis was carried out using custom-designed MATLAB software to measure the duration and depth of each current blockade events.

RESULTS

Membrane current characteristics

The total current through the membrane consists of both the nanopore current and the leakage current. The leakage current assumes a similar transport mechanism to the trap-assisted tunneling current (I_{tat}) through the gate dielectrics in semiconductor transistors. Figure 1 shows the measured current (open circle) through a pristine 10 nm thick SiN_x membrane. This leakage current is highly non-linear and strongly voltage-dependent. As a comparison, the intrinsic nanopore current (I_{np}, assuming 1 nm in size, filled squares) would have an ohmic behavior. It is clear from Figure 1 that I_{tat} dominates the measured current at high voltages (>3 V).

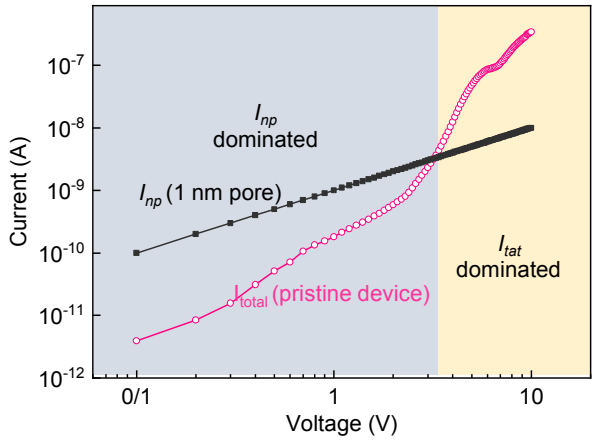

Figure 1: Leakage current in a pristine SiN_x membrane versus the calculated ohmic current through a 1 nm nanopore.

Figure 2 shows the representative time-traces of the total current for multiple SiN_x membranes exposed to various constant voltages. Three features were observed. The first is the random distribution of the leakage current baseline. The second is the leakage current could randomly decrease or increase before the breakdown events. The third is the temporary spike of the current, which does not necessarily correlate to a physical breakdown. This temporary spike could be ascribed to soft-breakdown or burst noise. These characteristics of the membrane current create a fidelity issue for CBD-based nanopore fabrication.

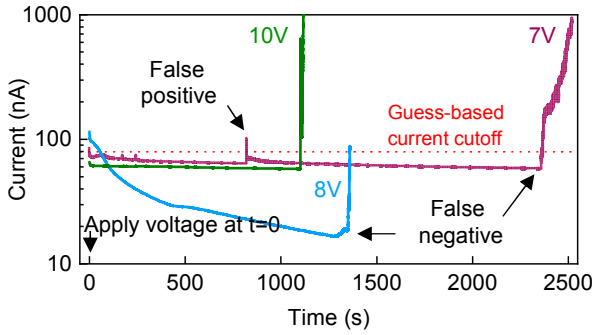

Figure 2: Leakage current time-traces at different DC voltages.

Membrane capacitance

Although stressing the membrane at a high voltage with certain duration and measuring the current at low voltage would solve the aforementioned interfering I_{tat} issue, the repetitive switching between high and low voltages on the capacitive membrane could result in transient phenomena. Inset of Figure 3 shows the simplified equivalent circuit model of the electrolyte-dielectric-electrolyte system before the breakdown. The membrane capacitance could lead to two fidelity issues. One is the actual voltage appearing across the membrane (V_{mem}) is hard to determine due to its RC time dependence. Figure 3 schematically shows the applied voltage and the V_{mem} as a function of time for two representative cases. The other issue is the interfering capacitive transient current ($I_{transient}$) when switching to a low voltage level for evaluating the I_{np}. A waiting time longer than RC is needed for $I_{transient}$ to be negligible leading to a long experimental time

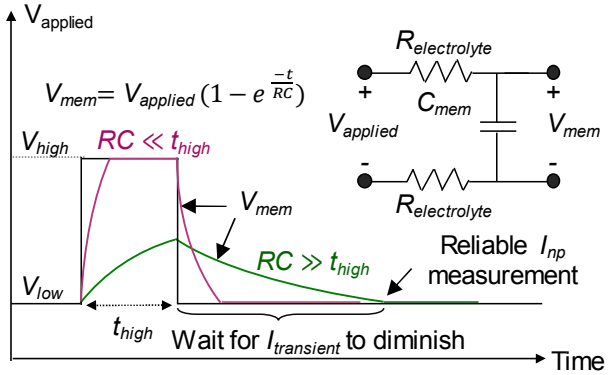

Figure 3: Membrane capacitance results in RC-dependent transmembrane voltage and transient current.

Moving Z-Score method

The moving Z-Score is an online adaptive learning algorithm for detecting the anomalous points in a time-trace. It measures the number of standard deviations each new observation is away from the mean over a pre-defined time window length of w.

Figure 4 shows an example of current time-trace with corresponding moving Z-Score (w=10). This anomalous

event could be easily detected by a Z-Score threshold. The moving Z-Score method alleviates the false negatives by not missing any suspicious events. Once a suspicious event is identified, subsequent confirmative test at low voltages is performed to avoid false positives. Figure 5 shows the overall fabrication flow-chart. A constant biasing voltage is applied to the membrane, and the moving Z-Score of the total current is monitored in real time. When an anomalous event with Z-Score>6 is detected, the high biasing voltage is immediately switched off to avoid possible nanopore enlargement. Subsequently, the membrane conductance is measured by IV scanning at low voltages to confirm if a nanopore is indeed formed. A positive nanopore formation would require triplicate measurements with conductance all bigger than a threshold of 1 nS and the IV curve linearity (r^2) all larger than 0.85. If the anomalous event turns out to be false positive, the constant biasing voltage will be reinitiated, and the process starts over again. If larger pores are desirable, this initial nanopore could be in-situ enlarged by applying electric fields.

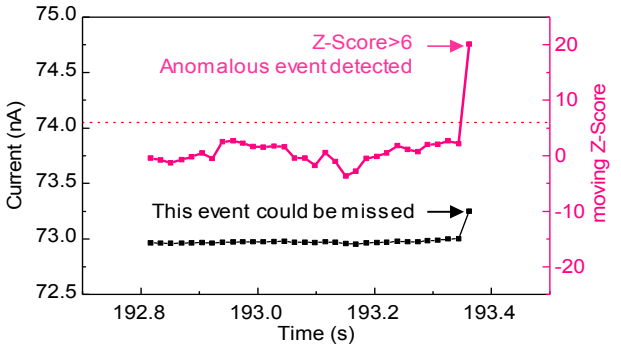

Figure 4: A representative time trace of measured current (black) and its moving Z-Score (pink).

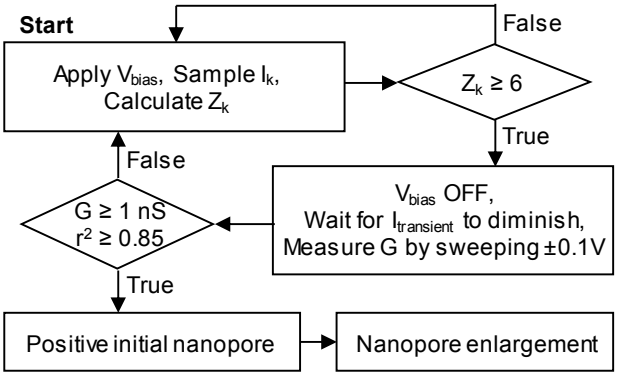

Figure 5: Flow-chart of the automated procedure for moving Z-Score based CBD nanopore fabrication.

High fidelity initial physical breakdown detection

High fidelity initial breakdown detection is critical to minimize the nanopore size. Figure 6 shows a representative case for a 10 nm SiN_x membrane under 8 V bias. Before the 8 V stress, the membrane conductance was examined by triplicate IV scanning between ± 0.1 V to confirm the assembly is leak-free and the SiN_x membrane is intact. Subsequently, the 8 V biasing was applied, the current was monitored, and the moving Z-Score was calculated in real time. The moving Z-Scores remain less than 6 during this random fluctuation phase until the first anomalous event detected. To examine if this event is true or false positive, IV scanning between ± 0.1 V was performed. As shown in the second enlargement view of Figure 6, a true positive breakdown would require the triplicate conductance and r^2 fall within the shaded area of the scattering plot. The IV scanning result rejected the anomalous event as a true positive. Therefore, the 8 V biasing was reapplied until the second anomalous event detected. The following triplicate IV scanning confirmed this event was a true positive.

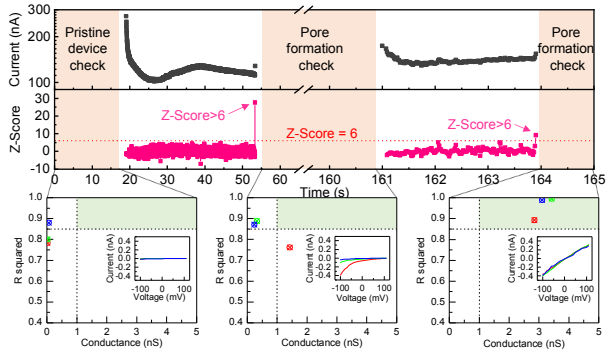

Figure 6: Top panel shows the current time-trace (black) under constant voltage stress with corresponding moving Z-Score (pink). Bottom panel shows scatter plots of membrane conductance and r^2 from the IV scannings.

Nanopore growth and enlargement kinetics

We studied the nanopore enlargement kinetics under various applied electric fields to accurately enlarge the nanopore to the desired diameter. The initial nanopore formed by the moving Z-Score method was subsequently subjected to multiple enlargement voltage pulses of 5 seconds in duration. Each pulse was followed by low voltage IV scanning to estimate the pore diameter after a sufficient waiting time. Figure 7 shows the nanopore diameter as a function of cumulative enlargement time for electric fields ranging from 0.4 V/nm to 0.8 V/nm applied to five individual initial nanopores. The diameter enlargement rate under each electric field could be derived from a linear fit. When subjected to 0.4 V/nm, the enlargement rate is 0.006 nm/s, whereas the electric field of 0.8 V/nm could lead to enlargement rate of 2 nm/s. From a practical perspective, there is a trade-off between sub-nanometer accuracy and total enlargement time.

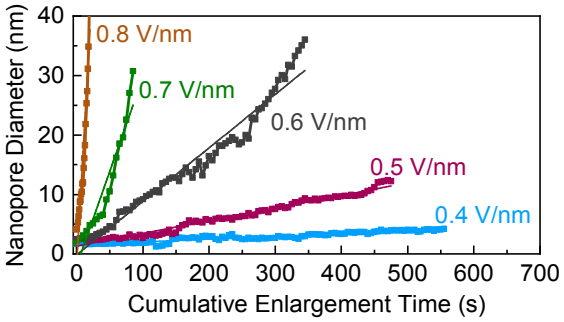

Figure 7: Nanopore diameter as a function of the cumulative enlargement time at various electric fields.

To understand the dependence of the diameter enlargement rate as a function of the electric field, we

model the nanopore as a cylinder with time-dependent diameter D(t). The instantaneous power dissipation within the nanopore is $P(t)=(G(t)V)^2$, where G(t) is the dynamic conductance. Without considering the accessing resistance, G(t) is determined by the nanopore area $\pi(D(t))^2/4$. Thus the instantaneous power dissipation P(t) within the nanopore would be proportional to $(D(t))^2$. As shown in Figure 8, a quadratic relationship between the pore area enlargement rate and the instantaneous nanopore diameter was observed. This strongly indicates the Joule heating is responsible for removing the material mass along the nanopore.

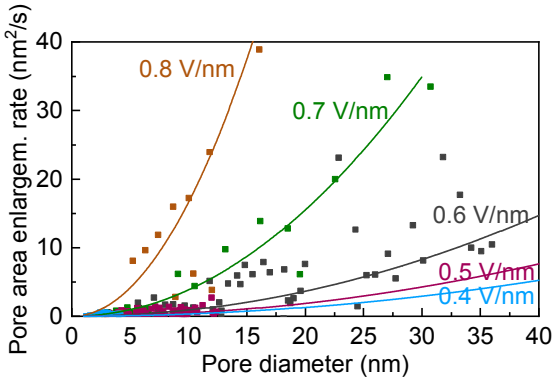

Figure 8: The pore area enlargement rate as a function of the instantaneous nanopore diameter for these devices.

Deterministic nanopore size

As shown in Figure 9, the moving Z-Score method could result in initial nanopore conductance tightly distributed within 3±1 nS with 100% success rate, which corresponds to the pore size of 1.7±0.6 nm. Nevertheless, the pore formed in this initial phase is usually too noisy to be practically used as a single molecule sensor. Therefore, we employ the enlargement process to tune the pore to a bigger size. Due to the fine enlargement rate of 1.26 nm/min at 0.5 V/nm, the initial nanopore size could be deterministically enlarged to a predefined size larger than 3 nm within the sub-nanometer resolution (Figure 9).

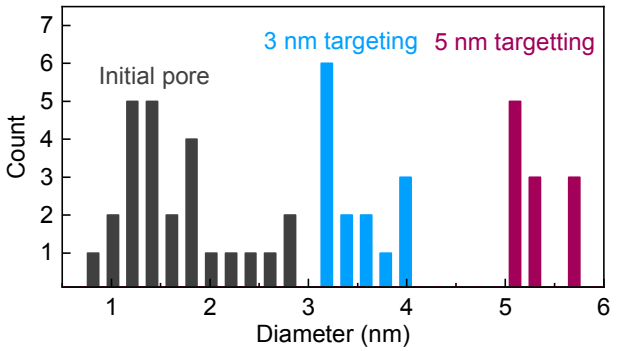

Figure 9: Distribution of nanopore size immediately after the initial breakdown and after fine size tuning by targeting 3 nm and 5 nm in diameter.

Single DNA molecule detection

To demonstrate the sensing performance of the fabricated nanopores, we performed translocation experiments using 48.5 kbp double-stranded λ-DNA. A nanopore of 9 nm diameter was prepared by the moving Z-Score method and the subsequent fine enlargement. Figure 10 shows a representative time-trace of current at 200 mV bias. The magnified view shows the ionic current blockade (*ΔI*) during the DNA molecule translocation. The inset of Figure 10 shows the current blockade distribution for a total number of N=604 events. The conductance change obtained for the translocation events (ΔG~1.6 nS) are in agreement with a 2.2 nm λ-DNA occupying a cylindrical nanopore. The average dwell time is 6.0 ms which reflects the translocation speed of λ-DNA molecule. We did not observe multiple current blockade levels that would indicate the translocation with a folded structure. This could be due to the noise overwhelming the short-lived folded events, although, the exact reason is unknown.

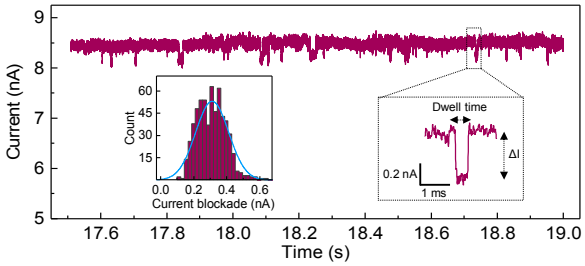

Figure 10. A representative current time-trace during the passage of DNA molecules through a nanopore.

CONCLUSIONS

In summary, we reported a robust moving Z-Score method for high fidelity nanopore fabrication. By online adaptive learning about the leakage current, false negatives can be alleviated by timely detection of the earliest physical breakdown event without setting an arbitrary cutoff current. The initial nanopores could be enlarged to a predefined diameter larger than 3 nm. Our studies show that Joule heating is the dominant mechanism for nanopore enlargement. The robust moving Z-Score method has the potential to minimize the CBD fabrication variation and increase the nanopore yield for the continued development of solid-state nanopore sensing applications.

ACKNOWLEDGMENTS

This project was supported by a grant from the National Science Foundation, USA (ECCS-1710831).

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